Vitamin D Metabolism: Biochemical, Physiological, and Clinical Considerations

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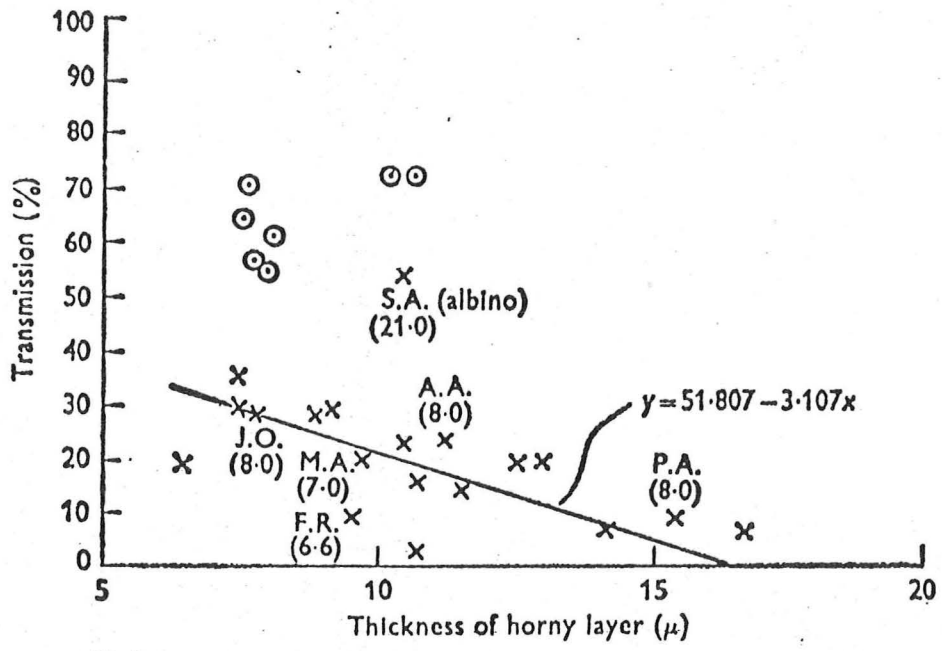
During the past decade, there has been an explosion of interest and research activity in the area of vitamin D metabolism. This activity has been aroused by disclosures which have enlightened our understanding of vitamin D action, and by discovery of vitamin D "metabolites" of major therapeutic potential. Thus, it is now known that vitamin D itself is biologically inactive, since the actions normally attributed to it - stimulation of intestinal calcium absorption and of bone resorption - are the result of its certain hydroxylated derivatives. One of the metabolites has been relegated a hormonal status, and the kidney, the site of its synthesis, has received another claim as an endocrine organ. My objectives today are to review: first the biochemistry of vitamin D metabolism; second, the physiological function of vitamin D (analogues and metabolites), and third, the role of vitamin D in the pathogenesis and treatment of various disorders of mineral metabolism, such as osteomalacia, renal osteodystrophy, and hypoparathyroidism.

Historical Background

Vitamin D has played an important role in the evolution of human races[1] and more recently in the ecology of certain fishes and birds. The absorption of μV light by skin, required for the synthesis of vitamin D_3 from 7-dehydrocholesterol, is dependent on skin pigment (Fig. 1). Lighter the skin, greater are the μV transmission and vitamin D_3 production. Thus, the skin pigment serves as a barrier for endogenous vitamin D_3 productiom. Near the tropics, it has been estimated that an untanned Caucasian adult subject would synthesize up to 800,000 units of vitamin D in a 6-hr period of exposure to sunlight. On the other hand, a deeply pigmented black subject would synthesize 5 to 10 per cent as much. Thus, his production



Variation of transmission of solar ultraviolet light (3000 to 4000 angstroms) through the stratum corneum, plotted against thickness of this layer. ⊙, Europeans; ×, Africans. The numbers in parentheses after initials are percentages for reflectance of blue light on the forearm.

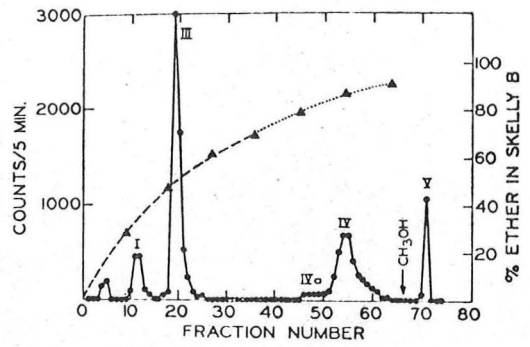
Fig. 1

would have been in the "safe" range, unlike that of his Caucasian counterpart. In contrast, north of 40°N or areas of little or indirect sunlight, the vitamin D production by the Caucasian subject may have been adequate, but that by the black insufficient. It has been suggested that the early hominids originating in the tropics were black, because white skin was selected against by predisposition to vitamin D toxicity. As they migrated north toward Europe, members with light skin prevailed, since those with deeply pigmented skin were selected against by predisposition to vitamin D deficiency. Thus, the white race may owe its origin to the requirement for vitamin D. While this theory may not seem entirely plausible, it may have a modern counterpart in the plight of Asiatic Indians who have migrated to England. While persisting in their customary dietary habits low in vitamin D content and living in a new land of reduced solar μV irradiation, some of them have developed osteomalacia or rickets[2].

In modern times, however, man has not usually depended solely on endogenous vitamin D production for his needs. The therapeutic use of exogenous vitamin D on a rational basis probably dates back to the classical studies of Mellanby[3] in 1919, showing that rickets could be prevented or cured with cod-liver oil. Later, McCollum et al. identified the antirachitic factor in cod-liver oil as vitamin D[4]. The discovery of cod-liver oil as a source of this important vitamin probably contributed to the virtual decimation of this once abundant species by overzealous fishing.

Ironically, vitamin D may play yet another role in modern ecology. The survival of many avian species, including terns and eagles, is threatened by exposure to pesticides, which cause thinning of shells. One of vitamin D metabolites, 25-hydroxycholecalciferol, may be able to overcome the effect of pesticides, and promote "hardening" of eggs.

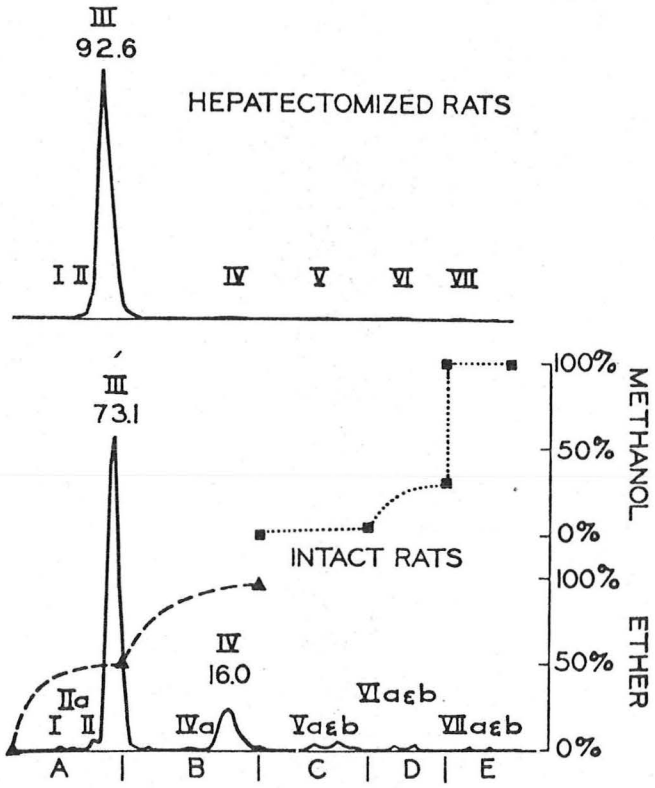
Biochemistry of Vitamin D



Silicic acid chromatography of vitamin D and its metabolites. Peaks III and I are vitamin D and its esters respectively, peaks IVa and V are unidentified, and peak IV is 25-hydroxychlolecalciferol.

Fig. 2

The preparation by Norman and DeLuca in 1962 of tritiated vitamin D_3 of high specific activity ushered in the current era of vitamin D research[5]. When tritiated vitamin D_3 was given in physiological amounts to rachitic rats, it was converted into several polar metabolites (Fig. 2). This figure shows the silicic acid chromatography of lipid extract of plasma after tritiated vitamin D_3 administration. Peak III represents vitamin D_3. Peak IV has been identified as 25-hydroxycholecalciferol (25-HCC). Peak V is 1,25-dihydroxycholecalciferol (1,25-DHCC).



Column profiles or radioactivity from the plasma of either control or hepatectomized rats 4 hr after they had received a 0.25 μg dose of ^{3}H-vitamin D_3.

Fig. 3

The major metabolite in plasma is 25-HCC, which is formed predominantly in the liver by hydroxylation at the C-25 position via a microsomal enzyme[6-8]. Thus, in hepatectomized rats, peak IV or 25-HCC disappears from plasma (Fig. 3).

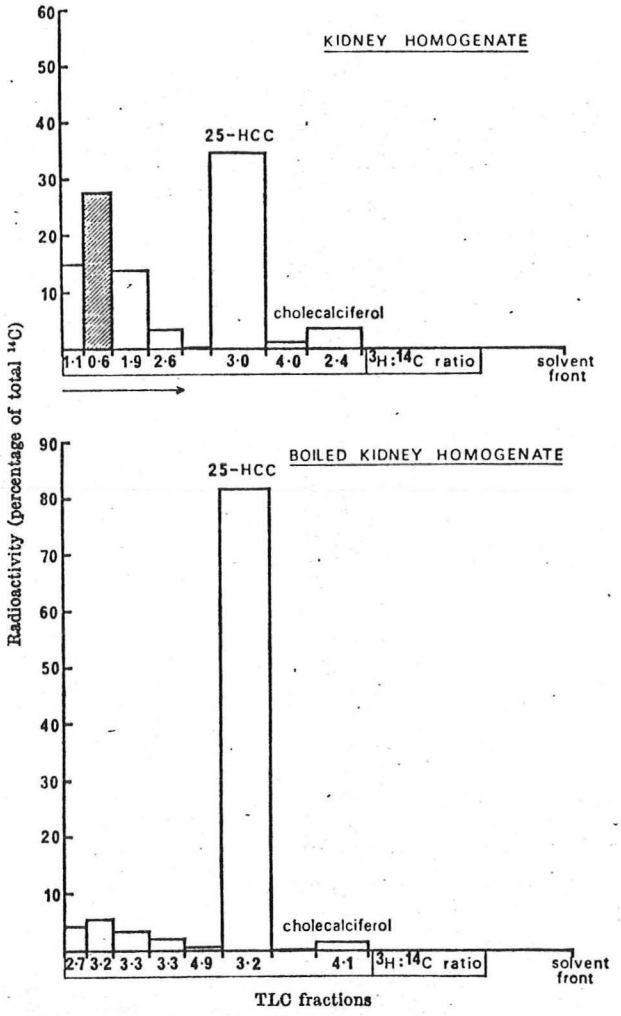
After it is formed in the liver, 25-HCC is transported to the kidney where it undergoes a second hydroxylation at the C-1 position to form 1,25-DHCC. Fraser and Kodicek were first to show that the production of 1,25-DHCC occurs in the kidney[9] (Fig. 4). When they incubated 4-^{14}C,1-^{3}H labelled 25-HCC with kidney homogenate, a ^{14}C peak deficient in tritium appeared (shown by shaded areas); this was later shown to be due to hydroxylation of C-1. The enzyme system for 1-hydroxylation has been shown to be localized exclusively in the mitochondrial fraction of the renal cortex[10]. This metabolite represents the major component present in the intestine and is also present in bone.

Other metabolites of vitamin D have been characterized, including 24,25-dihydroxycholecalciferol, 25,26-dihydroxycholecalciferol, and 1,24,25-trihydroxycholecalciferol[11]. Functional role of these metabolites has not been conclusively established. Figure 5 summarizes the metabolism of vitamin D.

Functional and Physiological Role of Vitamin D

Biological actions of vitamin D. Two actions of vitamin D are clearly recognized: the stimulation of intestinal calcium absorption and of skeletal resorption.

The response to vitamin D requires a period of 14 hours or more before intestinal calcium transport is stimulated. It is now known that this delay is the result of time required for conversion of vitamin D_3 to its active metabolites, and for transmitting new genetic information necessary for the biologic response. The time lag before a response in intestinal calcium transport is somewhat shortened by 25-HCC[12] (Fig. 6). However, 1,25-DHCC (peak V) causes an even more prompt stimulation of calcium absorption.



Thin layer radiochromatogram of $CHCl_3$-methanol extracts from incubation of [4-^{14}C,1-^{3}H] 25-HCC with vitamin D deficient chick kidney homogenate. Silica gel G thin layers were developed with ethyl acetate–heptane (1 : 1) to a 17 cm solvent front. ^{3}H : ^{14}C specific radioactivity ratios are given for each fraction. The shaded peak represents the ^{14}C of the tritium deficient metabolite.

Fig. 4

Both 25-HCC and 1,25-DHCC are capable of stimulating bone resorption in tissue culture[13] and in vivo[14]. This action does not require the presence of PTH. However, 1,25-DHCC is approximately 100 times more potent in eliciting this response than 25-HCC (Fig. 7), while vitamin D_3 is without effect in vitro. Thus, the biological activity of vitamin D resides in its two metabolites -1,25 DHCC and 25-HCC. Vitamin D_3 has no biological activity on its own; its activity is indirect via its metabolites. 25-HCC probably exerts most of its action by converting to the more active metabolite, 1,25-DHCC. However, it has some biological action of its own.

Other actions of vitamin D. Vitamin D administration increases intestinal absorption of phosphate, probably secondary to Ca absorption. The stimulation of bone mineralization, sometimes seen with vitamin D therapy, has been usually attributed to the rise in serum Ca and P resulting from vitamin D administration.[15] However, there is some evidence that vitamin D metabolites stimulate mineralization and osteoblastic bone formation, independently of changes in serum Ca and P.[16]

The renal effects of vitamin D metabolites depend on the species studied. In thyroparathyroidectomized dogs, 25-HCC and 1,25-DHCC increase renal tubular reabsorption of calcium and phosphorus.[17,18] In rats, however, 25-HCC enhances tubular reabsorption of phosphorus, only in the presence of either endogenous or exogenous PTH, and not in its absence.[19] In thyroparathyroidectomized human subjects, vitamin D treatment does not alter renal clearance of calcium.[20]

Mechanism of vitamin D-mediated intestinal Ca absorption. The sequence of events by which 1,25-DHCC induces calcium transport in the intestinal cell has been delineated by Haussler[21] (Fig. 8). First, 1,25-DHCC is bound to a protein receptor in the cytoplasm.

Flow diagram of probable events in metabolism of vitamin D.

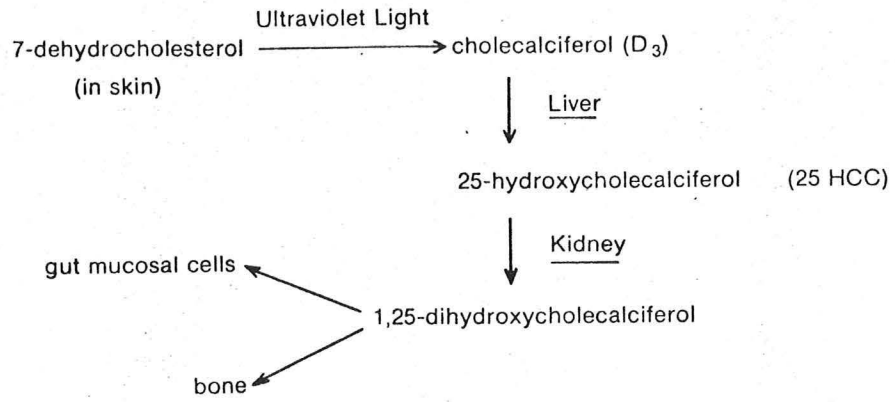


Fig. 5

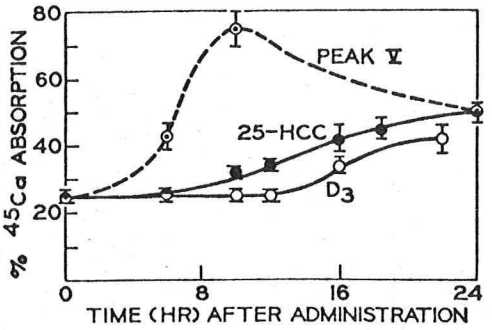
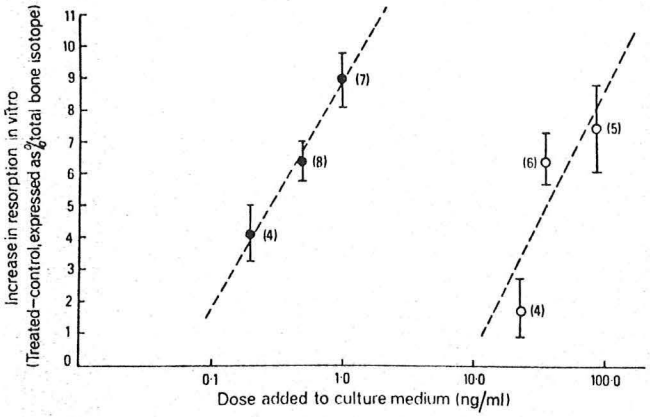


FIGURE 6: Response of chick intestinal calcium absorption to 25-HCC, vitamin D_3, and peak V (1,25-DHCC). Each point represents the average of 4–7 chicks ± standard error. (●———●) 325 pmoles given iv; (○———○) 325 pmoles given iv; (⊙———⊙) 325 pmoles administered orally.



Direct *in vitro* comparison of the response of bone explants to $1,25\text{-}(OH)_2D_3$ (-- ●--) and $25\text{-}OHD_3$ (--○--). Paired half-calvaria from six-day-old mice prelabeled with ^{45}Ca were treated with or without metabolite at the indicated doses for 48h. The response is plotted as the increase in resorption, expressed as the difference, treated half—control half, in % release of bone isotope into the medium. The points are means ± the standard error, and the numbers of pairs of explants at each dose (logarithmic scale) are shown in parentheses. The dose-response lines were computed by the method of least squares

Fig. 7

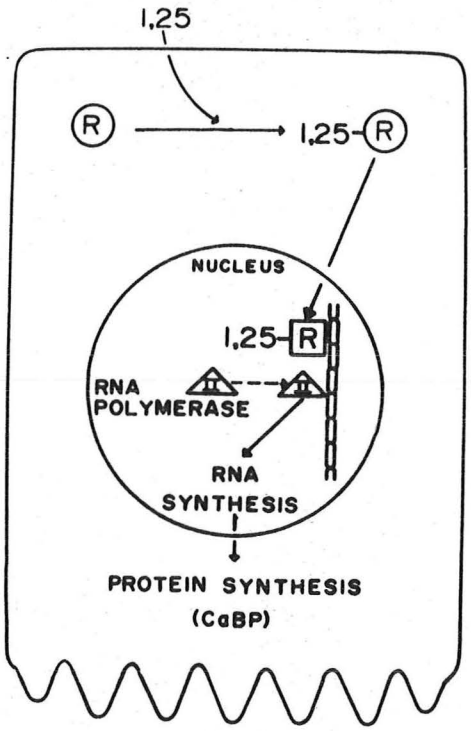


Fig. 8

The complex of 1,25-DHCC and cytoplasmic receptor is then bound to the nuclear chromatin. This receptor association is highly specific for 1,25-DHCC (Fig. 9). There it stimulates RNA polymerase II[22], leading to the synthesis of messenger RNA and protein (presumably Ca binding protein). Calcium transport then ensues. Thus, the association of 1,25-DHCC with the chromatic fraction of the nucleus preceeds the stimulation of the physiologic response (Fig. 10). These responses to 1,25-DHCC may be blocked by actinomycin D[23], an inhibitor of DNA-directed RNA synthesis.

Regulation of the synthesis of 25-HCC and 1,25-DHCC. In the chick, no regulatory mechanism for the conversion of vitamin D_3 to 25-HCC could be found when intake of vitamin D_3 was varied within the physiologic range[24]. Similiarly in man, increased amounts of 25-HCC appears in plasma following therapeutic dosages of vitamin D_3 (Fig. 11)[25]. The plasma concentration of 25-HCC is not significantly correlated with circulating concentrations of Ca, P or immunoreactive PTH (Fig. 12).

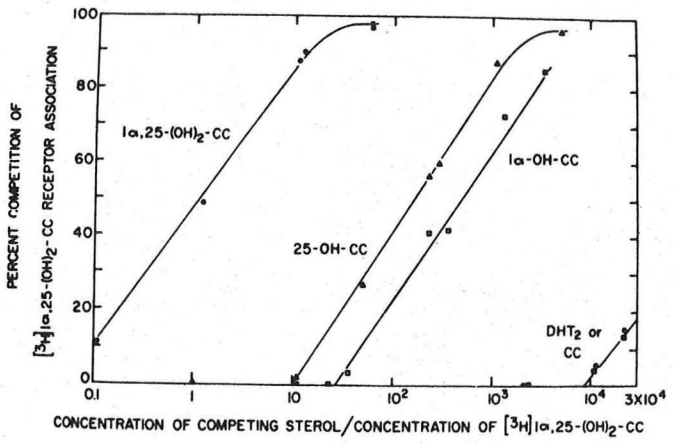


Fig. 9

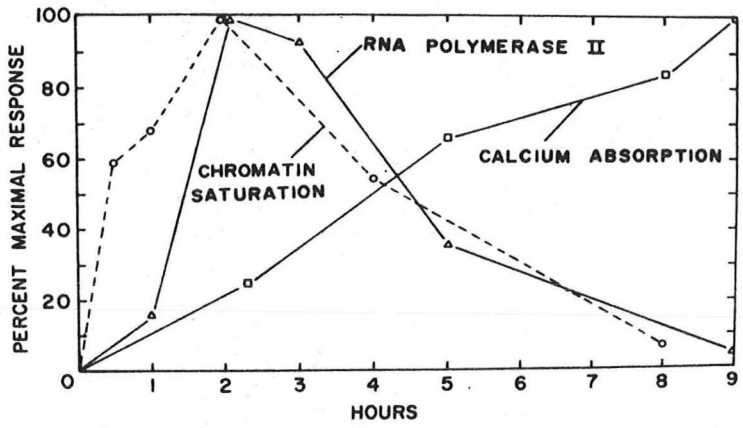
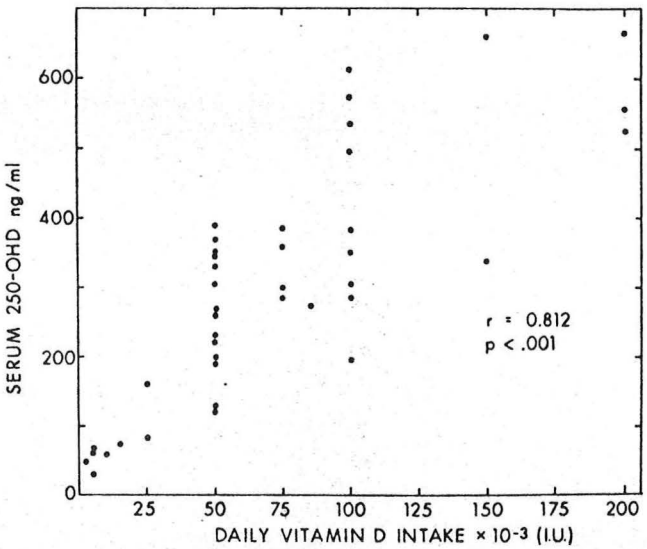


Fig. 10

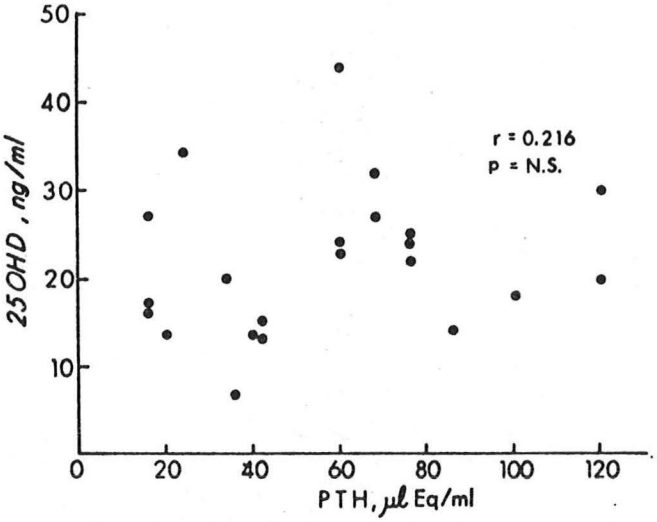


Serum 25-OHD plotted as a function of daily dose of vitamin D (long-term treatment) in 41 patients with hypoparathyroidism or sex-linked hypophosphatemic rickets.

Fig. 11

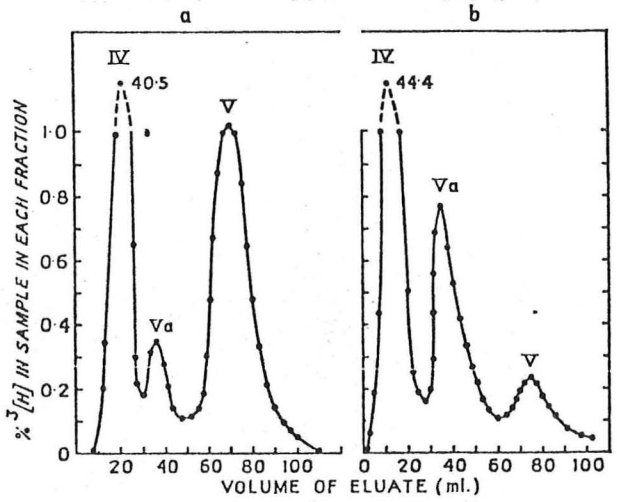
Thus, there is no evidence suggesting regulation of the production of 25-HCC in chick and man.

However, the activity of 25-HCC-1-hydroxylase is apparently subject to regulation depending on Ca needs of the organism. Unfortunately, many conflicting reports have appeared implicating different mechanism for the regulation of 1,25-DHCC synthesis. It has been shown that a low Ca diet stimulates the synthesis of 1,25-DHCC, and suppresses that of 24,25-DHCC, while a high Ca diet exerts the opposite effects[15]. Since parathyroidectomy in the chick reduces the circulating levels of 1,25-DHCC, it has been suggested that PTH may be the trophic hormone for 1,25-DHCC synthesis[26]. However, another laboratory reported inhibition of 1,25-DHCC synthesis by PTH, using large amounts of the peptide[27] (Fig. 13). Low phosphorus diet has been reported to stimulate the synthesis of 1,25-DHCC.[28] Thus, it has been proposed that PTH may mediate the synthesis of 1,25-DHCC by altering the intracellular concentration of phosphate[29]. However, in another study, phosphorus deprivation failed to alter vitamin D



Relationship of serum 25-OHD concentration to serum iPTH concentration in normal children.

Fig. 12



—Experiment I: chromatography on LH20 sephadex of serum extracts from D-deficient rats 8 hours after tritiated 25-H.C.C.

(a) Control rats; (b) rats receiving 200 I.U. parathyroid extract per 24 hr.

Fig. 13

metabolism[30]. In one study[31], PTH and cyclic AMP stimulated the synthesis of 1,25-DHCC, while in another study, no stimulating effect of PTH was found.[32]

The major difficulty with these studies is that each one of these experimental manipulations is accompanied by simultaneous changes in a number of parameters. For example, a low Ca diet will probably stimulate parathyroid function, and may affect serum and intracellular concentrations of Ca and P. There is some evidence supporting the concept that the concentration of calcium in the mitochondria of the renal tubular cell, where 1-hydroxylation takes place, may be critical for the synthesis of 1,25-DHCC. In a preparation of kidney homogenate, McIntyre has shown stimulation of 25-HCC-1-hydroxylation when the Ca concentration of the incubating medium was low, and suppression in medium of high Ca content[33]. PTH, implicated by some to stimulate 1-hydroxylase, augments the efflux of Ca^{++} out of kidney mitochondria[34]. Diphosphonate and 1,25-DHCC, which have been shown to inhibit 1-hydroxylation, reduce the efflux of Ca^{++}. The resolution of this problem will probably require a better restriction of experimental variables, and the determination of intracellular (intramitochondrial) content of Ca (and P).

Therapeutic Considerations

Available and investigational drugs. The commercially available vitamin D is vitamin D_2 or ergocalciferol. The native vitamin, cholecalciferol or vitamin D_3, is no longer available today (Fig. 14). The two vitamins possess similar biological activity (40,000

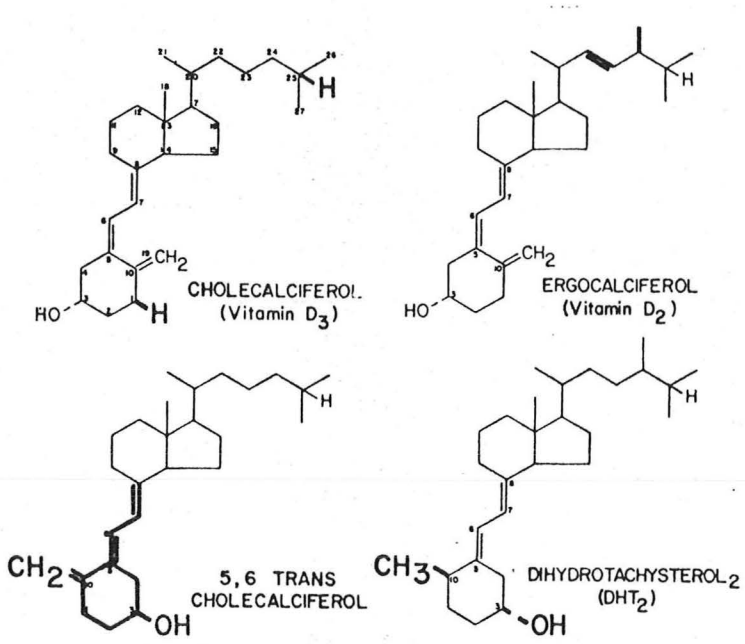


Fig. 14

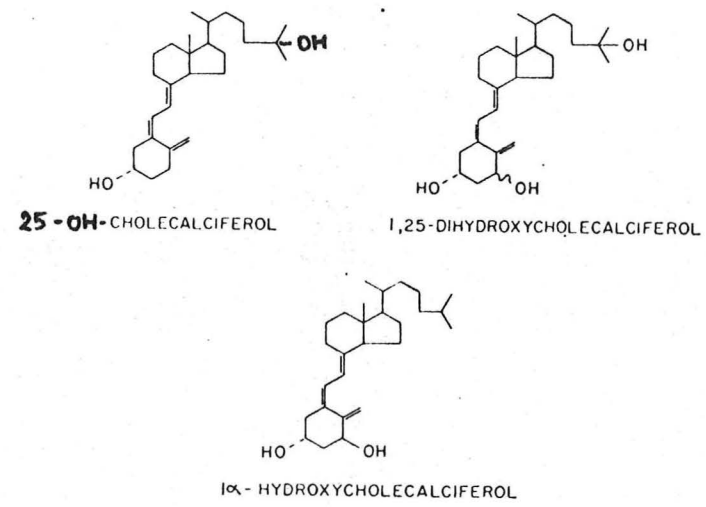


Fig. 15

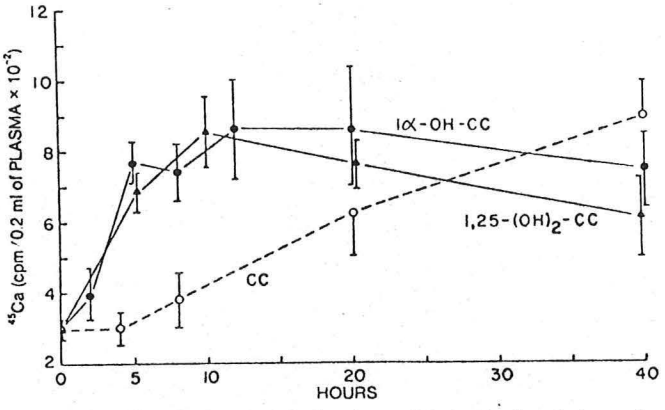
units/mg). Another commercial preparation is dihydrotachysterol (DHT). Unlike Hytakerol, an impure older preparation, dihydrotachysterol is now available in a pure form in 0.2 mg tablets. It is more active biologically than vitamin D_3 (0.4 mg equivalent to 1 mg for vitamin D_3). This higher activity is believed to be due to trans configuration at C5-C6 of the A ring. Thus, 3β-hydroxyl group of DHT is stereochemically similar to the 1α-hydroxyl of 1,25-DHCC.

Three metabolites of vitamin D are available on an investigational basis (Fig. 15). 25-HCC is available in 20 μg and 50 μg capsules. It is approximately 10-20 times more potent than vitamin D_3 (50-100 μg equivalent to 1 mg of vitamin D_3). 1,25-DHCC is virtually unavailable, but its synthetic analogue, 1-alpha-hydroxy-cholecalciferol (1-α-HCC) may be obtained through a personal investigational new drug application from the Food and Drug Administration. 1α-HCC has been shown to possess the same biological activity as that of 1,25-DHCC. Thus, the time course of induction of intestinal calcium transport by this compound is just as rapid as that of 1,25-DHCC (Fig. 16)[35]. It is approximately 500 to 1000 times more potent than vitamin D_3 *in vivo* (1 to 2 μg equivalent to 1 mg of vitamin D_3). The high biological activity of 1α-HCC can be ascribed to its conversion to 1,25-DHCC by 25-hydroxylation in liver when it is given *in vivo*[36]. 1α-HCC has a much lower activity *in vitro* (comparable to that of 25-HCC), attesting to the need for 25-hydroxylation *in vivo* to achieve full biological activity.

Oral calcium supplementation is usually provided with vitamin D therapy.

Oral Calcium Preparations

Name	Form	Size	Ca Content	1 g Ca equivalent
Ca gluconate	tablet	1.0 g	89 mg/tab	11 tab
Ca lactate	tablet	0.3 g	39 /tab	26 tab
Ca lactate	tablet	0.6 g	78 /tab	13 tab
OsCal	tablet	one size	250 /tab	4 tab
NeoCalglucon	~~tablet~~ liquid	———	92 /4ml	43 ml



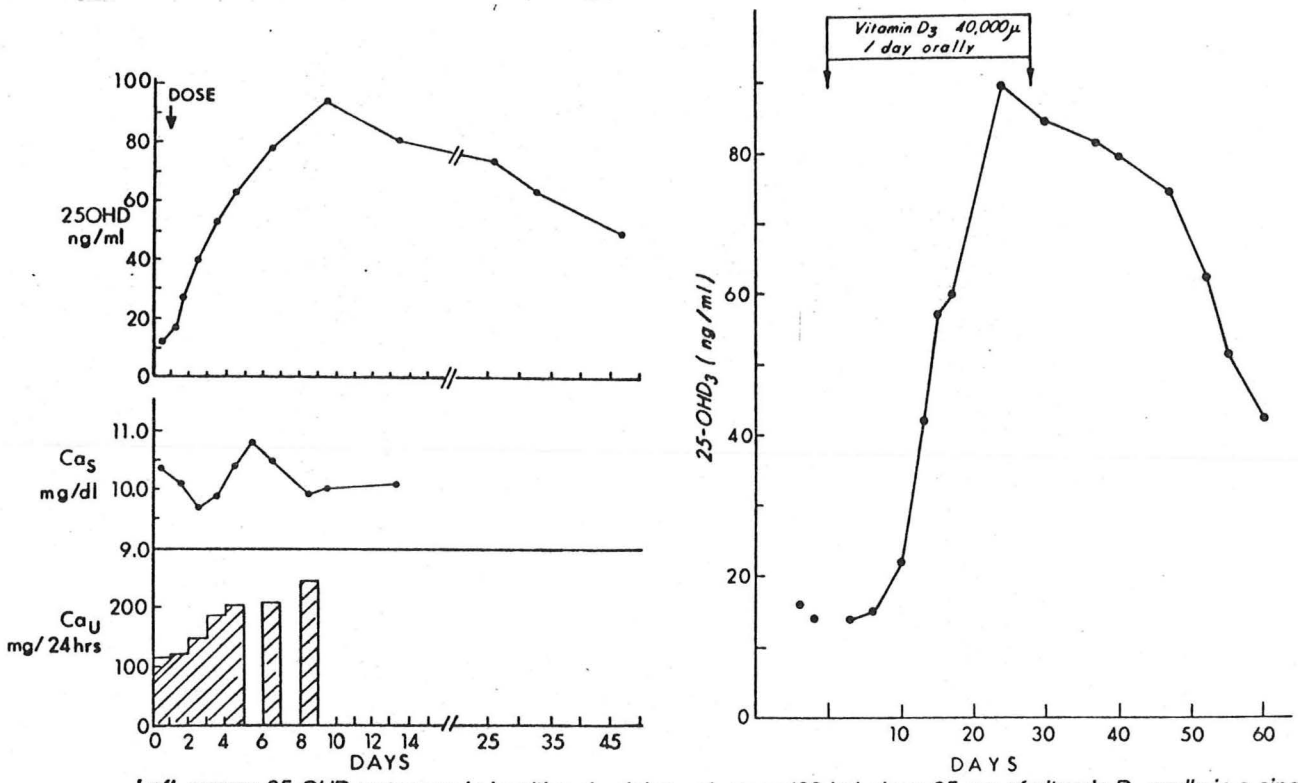
Time-course of induction of intestinal calcium absorption in rachitic chicks after an oral dose of 0.5 nmol of CC (O - - - O), 1α-OH-CC (●—●), or 1,25-$(OH)_2$-CC (▲--▲). Calcium absorption was assayed as described in *Methods*. Each number represents the average of five separate animals ± SD.

Fig. 16

Calcium supplements should be given in divided doses, at least three times a day, since the absorption of calcium from the same total intake of calcium is greater when the load is divided[37].

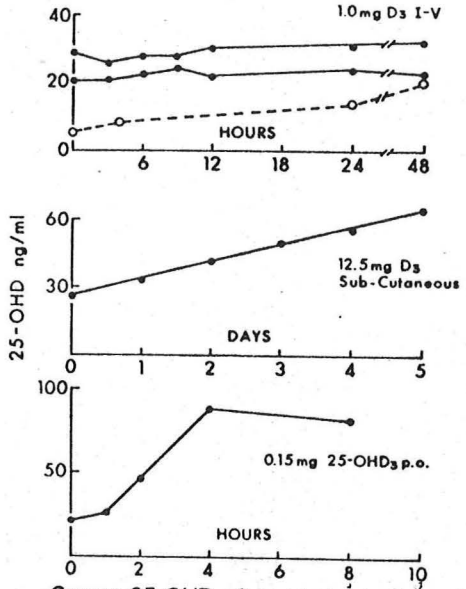
Turnover rates of vitamin D and metabolites. Rational therapy with vitamin D requires an appreciation of turnover rates for various calciferol steroids. The mean plasma half-life for the biologically-inactive vitamin D_3 is approximately 4.5 days[38]. This rapid clearance probably reflects assimulation by liver for 25-hydroxylation. From the therapeutic standpoint, it is probably more useful to consider the turnover of 25-HCC, the biologically-active metabolite, after the oral administration of vitamin D_3. Following an oral administration of a single, very large dose of vitamin D_3 (25 mg or 1 million units), an increase in plasma concentration of 25-HCC occurs within 2 days (Fig. 17)[25]. However, when vitamin D_3 is given in a more customary schedule (40,000 units or 1 mg daily), there is a lag of nearly 10 days before plasma concentration of 25-HCC increases. Upon stopping vitamin D_3, plasma 25-HCC declines slowly, with $t_{1/2}$ of nearly 25 days.

When 25-HCC is given orally, it appears in plasma very rapidly, reaching a peak at around 4 hours (Fig. 18)[25]. The concentration achieved in plasma is directly dependent on the dose. It disappears from plasma with a $t_{1/2}$ ranging from 5-7 days. Thus, 25-HCC possesses therapeutic advantages over vitamin D_3, since it can provide the desired plasma level of 25-HCC more predictably, and since it has less



***Left,** serum 25-OHD response in healthy physician volunteer (69 kg) given 25 mg of vitamin D_3 orally in a single dose. **Right,** serum 25-OHD response in adult volunteer (70 kg) to 1 mg of vitamin D_3 given orally daily for 25 days.*

Fig. 17



*Serum 25-OHD after administration of vitamin D or 25-OHD. **Top,** intravenous injection of 1 mg of vitamin D_3 in two normal St. Louis adults (solid lines) and one healthy London adult (broken line). **Middle,** subcutaneous injection of 12.5 mg of vitamin D_3 in postgastrectomy patient. **Bottom,** oral administration of 25-OHD$_3$ (2.5 μg/kg) in healthy adult.*

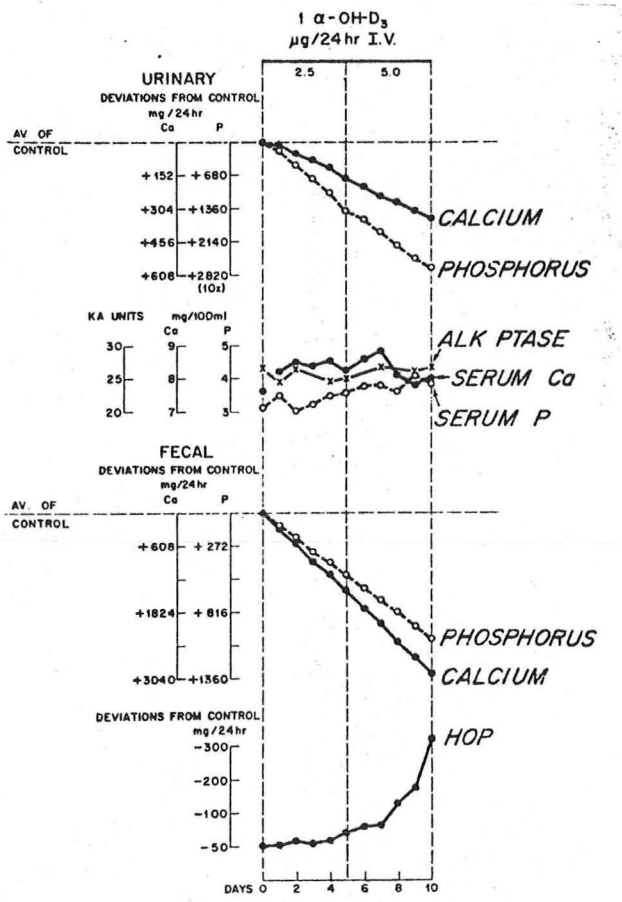
Fig. 18

problem with toxicity owing to its more rapid turnover.

The turnover rates of 1,25-DHCC and 1α-HCC are not as well known. After oral administration, they appear more rapidly in plasma than 25-HCC. It has been estimated that the $t_{1/2}$ for plasma disappearance of 1,25-DHCC is 1-10 days[39]. The kinetics of plasma appearance of 1,25-DHCC after 25-HCC or vitamin D_3 administration are not known.

Role of Vitamin D in Clinical Disorders

The clinical disorders of vitamin D metabolism may be classified into four groups: 1. reduced availability of vitamin D_3,



Effects of Daily Intravenous Administration of Crystalline 1α-Hydroxy Vitamin D_3 in a Dose of 2.5 μg for the First Five Days, Followed by a Dose of 5 μg for the Next Five Days, in Subject 3, with Osteomalacia.

Fig. 19

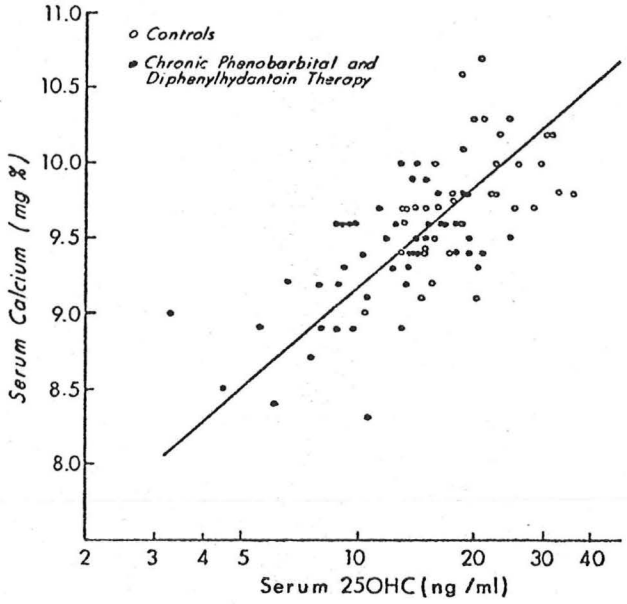
2. reduced availability of 25-HCC, 3. defective synthesis of 1,25-DHCC, and 4. altered tissue responsiveness to vitamin D action.

1. Reduced availability of Vit D_3
   a. Low μV exposure or uptake
   b. Dietary deficiency of Vitamin D
   c. Malabsorption syndrome: chronic pancreatitis, blind loop, biliary cirrhosis

I have already discussed the need for μV exposure to initiate endogenous production of vitamin D_3 at the beginning of this conference. Nutritional deficiency of vitamin D is rare in the United States, because of fortification of foods with the vitamin. The clinical presentation of vitamin D deficiency is osteomalacia or rickets. The intestinal absorption of calcium is reduced as the consequence of vitamin D lack. The secondary hyperparathyroidism[40], ensuing from reduced input of calcium from gut, probably accounts for the hypophosphatemia, by promoting renal P loss. Serum Ca is usually normal during early stages of the disease, but may fall in advanced stages. Urinary Ca is typically low. Bone disease, characterized by defective mineralization (an increase in non-mineralized matrix or osteoid), has been ascribed to the low plasma Ca x P product. Serum alkaline phosphatase is usually high.

Most patients respond to vitamin D 50,000 - 100,000 units/day orally and oral calcium supplements (equivalent to 1 - 1.5 g elemental calcium in 4 divided doses/day). Recently, Bordier has shown efficacy with low doses of 1α-HCC or 1,25-DHCC (2.5-5 μg/day) (Fig. 19)[41]. In our experience, 25-HCC at 20-50 μg/day may also be effective.

Occasionally, patients with osteomalacia may carry an erroneous diagnosis of osteoporosis. It is imperative that the two conditions are differentiated, since the response to therapy may be dramatic in osteomalacia, unlike in osteoporosis. The following features of osteomalacia (of vitamin D deficiency) may be helpful in the differential diagnosis. The patients with osteomalacia may complain of bone tenderness and muscle weakness. Pseudofractures may be present on skeletal roentgenogram. Serum P and urinary Ca are typically low.



. Correlation of Serum Calcium with Serum 25OHC in Patients Receiving Chronic Combination-Drug Therapy (r Equal to 0.56, p Less than 0.001) and Controls (r Equal to 0.47, p Less than 0.01) — for the Combined Populations (r Equal to 0.63, p Less than 0.001).

Fig. 20

2. Reduced Availability of 25-HCC
   a. ? Hepatic disease (cirrhosis)
   b. Anticonvulsant therapy
   c. Pesticide exposure
   d. ? Uremia

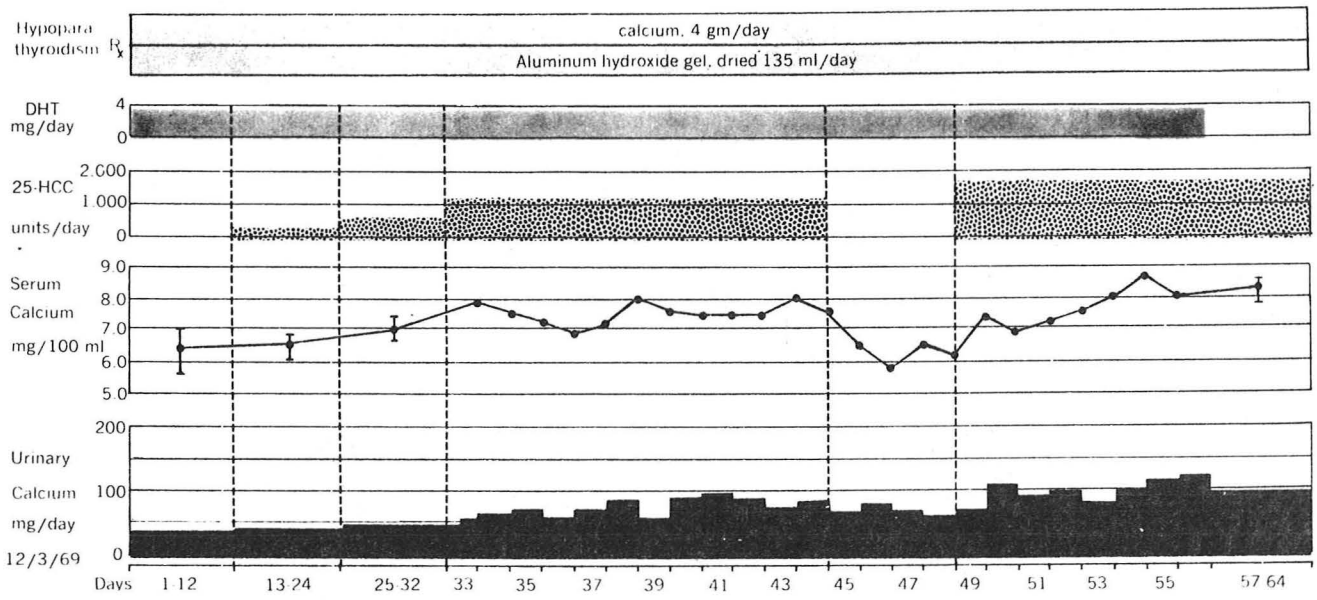
Hepner and Roginsky has reported reduced serum values of 25-HCC in patients with portal cirrhosis[42]. It is not known whether the decreased level of 25-HCC is the result of defective 25-hydroxylation of vitamin D_3 in the diseased liver, or of inadequate vitamin D intake associated with poor nutrition. Symptomatic osteomalacia in portal cirrhosis is rare.

Anticonvulsants (Dilantin and phenobarbital) may induce vitamin D deficiency by stimulating hepatic microsomal P-450 enzymatic activity[43]. This results in accelerated turnover of vitamin D and 25-HCC to more polar and inactive compounds and reduces the availability of 25-HCC, which may lead to lower production of 1,25-DHCC by the kidney. Indeed, low serum concentrations of 25-HCC has been reported in patients undergoing anticonvulsant therapy (Fig. 20)[43]. Intestinal calcium absorption may be low.[44] Occasional patients may develop symptomatic osteomalacia[45]. They may be successfully treated with relatively low doses of vitamin D (50,000 units twice a week orally). If patients with osteoporosis develop epilepsy and require anticonvulsant therapy, the prophylactic therapy with vitamin D is recommended.

Although reduced serum concentration of 25-HCC has been reported in uremia, it is probably due to low vitamin D content of the low protein diets prescribed for the renal disease.

3. Defective Synthesis of 1,25-DHCC
   a. Hypoparathyroidism
   b. Pseudohypoparathyroidism
   c. Vitamin D-dependent rickets
   d. Chronic renal failure
   e. Diphosphonate therapy

Most patients with hypoparathyroidism respond to 50,000 - 100,000 units (1.25 to 2.5 mg) of vitamin D/day, or to 0.4 to 0.8 mg of dihydrotachysterol/day. Oral calcium supplements (equivalent to 1 to 1.5 g elemental calcium/day) should be given. If hyperphosphatemia is present, a low P diet or P-binding antacid (for example, Gelusil



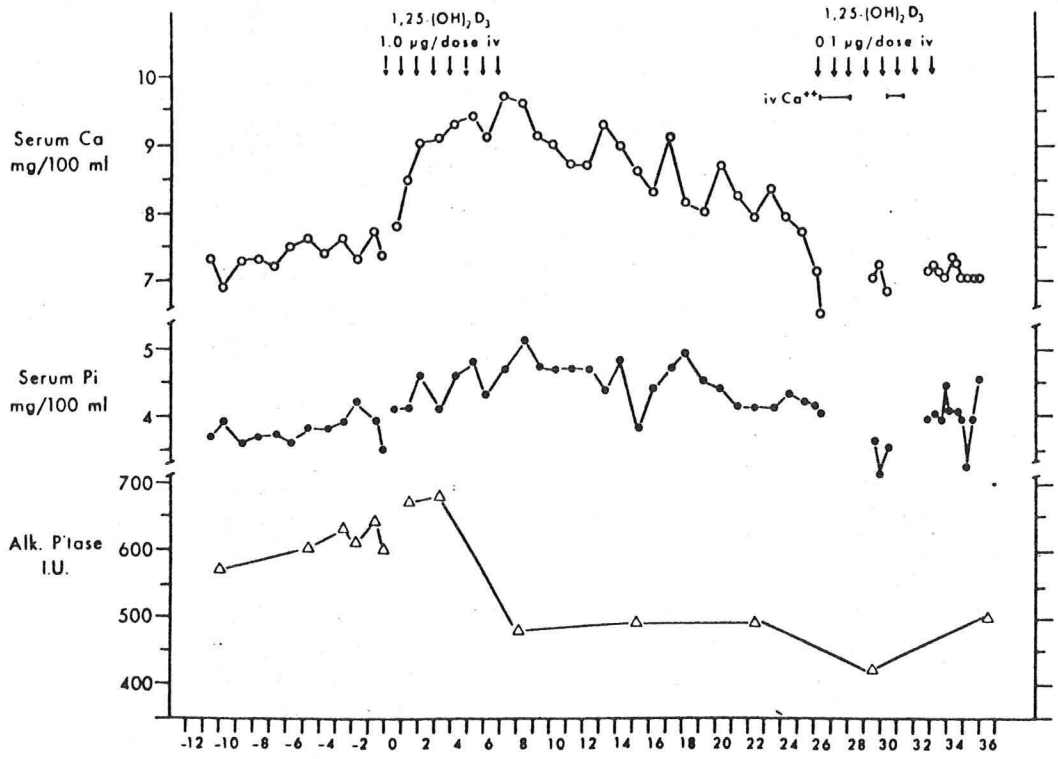
Effect of 25-HCC in patient 1 (first course) on serum and urinary calcium levels. During first 32 days of study, serum calcium concentration presented as mean ± range, and urinary calcium excretion as mean of each period.

Fig. 21

1 oz three times a day before meals) may be required.

However, occasional patients will need more than this dosage of the vitamin. The vitamin D resistance has been ascribed to an impaired synthesis of 1,25-DHCC, which is found in association with low plasma concentration of PTH, hypocalcemia or hyperphosphatemia. The defective 1-hydroxylation of 25-HCC may be acquired during treatment with 25-HCC (Fig. 21)[46]. This patient was hypocalcemic despite treatment with vitamin D of up to 1 million units/day and dihydrotachysterol (4 mg/day). Treatment with 25-hydroxycholecalciferol 1200 units (30 µg)/day increased serum Ca to a high of 8 mg%. Subsequently, she developed resistance to 25-HCC, even though the dosage of the drug was increased to 10,000 units (or 0.25 mg)/day. However, she responded to 1,25-DHCC and has been maintained successfully on a dosage of 2 µg/day.

Features of vitamin D dependent rickets are: autosomal recessive mode of inheritance, frequent association of hypocalcemia, parathyroid stimulation, and low or undetectable circulating concentration of 1,25-DHCC. While they may be resistant to vitamin D, they may respond to small dosage of 1,25-DHCC (1 µg/day) (Fig. 22)[47]. In contrast, features of familial vitamin D-resistant rickets are: sex-linked recessive mode of inheritance, normocalcemia (usually), normal parathyroid function[48], and normal or low circulating concentration of 1,25-DHCC. They do not respond to treatment with 1,25-DHCC (1 to 2 µg/day)[49]. It has been suggested[50] that there is no impairment in vitamin D metabolism, and that the primary abnormality

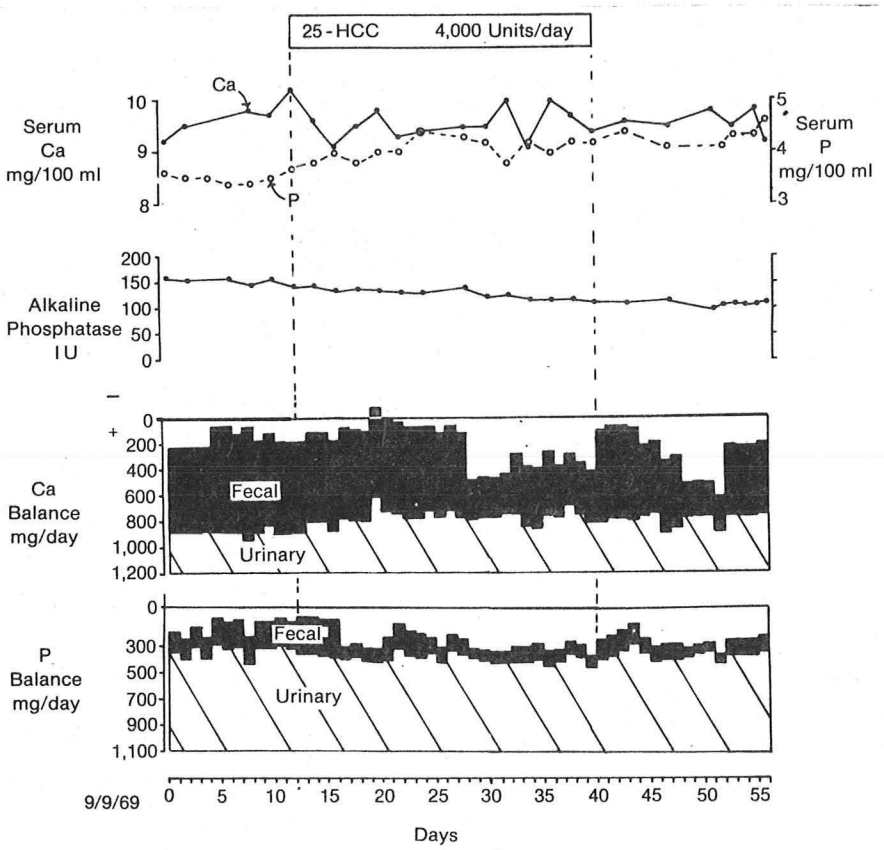


Serum Calcium (Ca) and Inorganic Phosphorus (Pi) Concentrations and Alkaline Phosphatase (Alk. P'tase) Activities in Case 5, Treated with α,25-$(OH)_2D_3$, 1.0 µg per Day on Zero to Seventh Days and 0.1 G per Day on 26th to 33d Days.

Fig. 22

is the defective renal tubular reabsorption (or intestinal absorption) of phosphorus. Osteomalacia presumably results from "P leak" rather than from vitamin D deficiency, unlike in vitamin D dependent rickets. However, contradictory evidence have appeared, demonstrating high serum immunoreactivity of PTH[51], amelioration of hypophosphatemia with calcium infusion[52], and responsiveness to 25-HCC (Fig. 23)[53] and to 1,25-DHCC (Fig. 24)[49] in vitamin D-resistant rickets. Thus, the presence of at least some degree of defective 1,25-DHCC synthesis cannot be excluded.

The most common cause of defective 1,25-DHCC synthesis is chronic renal failure. This finding is not unexpected since the kidney is the site of 1-hydroxylation. Low plasma concentration of 1,25-DHCC in renal disease has been directly shown by radioreceptor assay (Fig. 25)[35]. Thus, intestinal absorption of calcium is frequently low, particularly when it is tested by jejunal perfusion[54]. Histological evidence of osteomalacia may be demonstrated on bone biopsy. Brickman et al.[55,56] have reported successful treatment with 1,25-DHCC, at dosages of less than 3 µg/day. Treatment was followed by: increases in intestinal Ca absorption and serum Ca (Fig. 25), decreases in serum immunoreactive PTH and alkaline phosphatase activity, and on iliac crest biopsy of bone, an evidence for reduction of bone resorption and an improvement in mineralization.



Effect of 25-HCC in patient 1 (familial hypophosphatemia). Serum alkaline phosphatase activity is presented in international units (IU). Calcium and phosphorus balances are presented as follows: Intake is plotted downward from the zero line. Urinary Ca or P is represented by the hatched areas above the intake line. Fecal Ca or P is represented by the shaded areas above the urinary values. Positive balance is, therefore, indicated by clear areas below the zero line, negative balance by the shaded areas above the zero line.

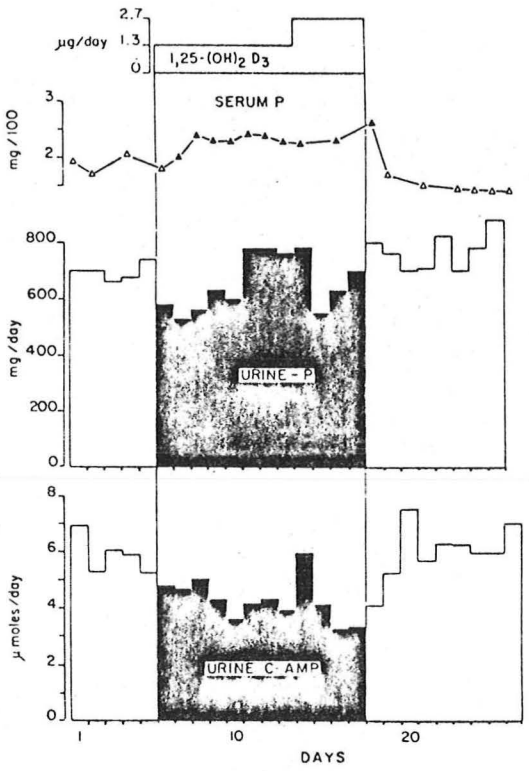
Fig. 23

Unfortunately, other workers have shown that 1,25-DHCC may stimulate bone resorption, even though it may improve mineralization[57]. Further, the dosage of 1,25-DHCC must be regulated carefully and P-binding antacids may be required to prevent the development of hypercalcemia and aggravation of hyperphosphatemia.

There is some evidence that 25-HCC may be efficacious in renal osteodystrophy. When it is given in sufficiently high doses (50 to 100 µg/day), the intestinal calcium absorption may increase and mineralization of osteoid may occur. These effects probably reflect direct action of 25-HCC.

Of the commercially available vitamin D preparations, dihydrotachysterol (0.2 to 0.4 mg/day) has been shown to be useful in the treatment of renal osteodystrophy[58]. The drug is usually given with oral Ca supplements (equivalent to 1 to 1.5 g elemental Ca/day) and P-binding antacids. The response to vitamin D_2 or vitamin D_3 even in large dosages, has been variable. The basis for the greater biologically activity of dihydrotachysterol is probably the presence of "pseudo 1-hydroxyl group", as mentioned before.

Diphosphonate (EHDP) may interfere with 1-hydroxylation of 25-HCC, presumably by affecting calcium transport of the renal mitochondria[34]. Defective synthesis of 1,25-DHCC has been shown in rats treated with EHDP (Fig. 27)[59]. The intestinal Ca absorption, shown to be low in EHDP-treated rats, may be corrected by the administration of 1,25-DHCC (Fig. 28)[60]. However, these effects were produced with amounts of EHDP which were 50-100 fold those given to man for the treatment of ectopic calcification. In our experience,



Serum Phosphorus (P) Concentrations and Urinary Excretion Rates for Phosphorus and Cyclic Adenosine Monophosphate (c-AMP) in Case 2, Treated with 1,25-$(OH)_2D_3$.

Fig. 24

the intestinal absorption of calcium is not reduced in patients with nephrolithiasis who received EHDP at a dosage of 20 mg/kg/day orally.

4. Altered Tissue Responsiveness to Vitamin D Action
   a. Steroid therapy
   b. Sarcoidosis
   c. ? Absorptive hypercalciuria

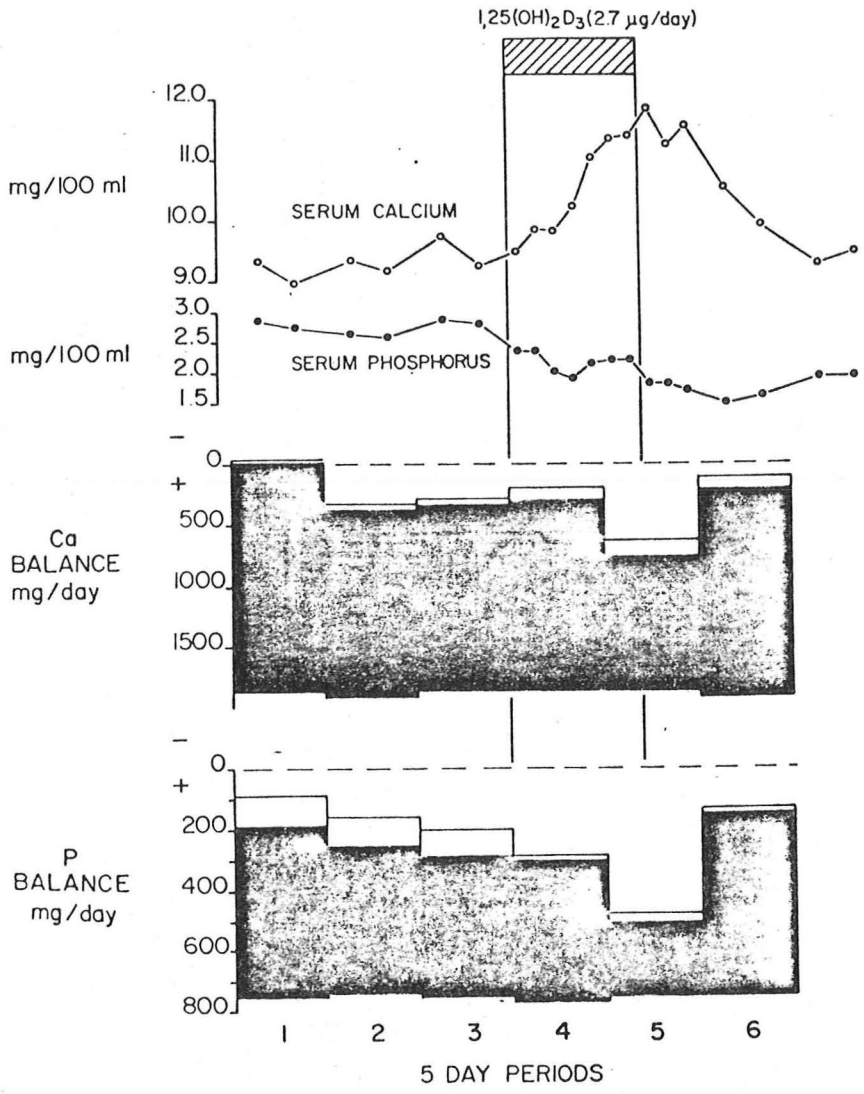
Carbohydrate-active steroids, such as prednisone, are known to inhibit vitamin D-induced stimulation of intestinal calcium absorption[61,62] and bone resorption[63]. It has been shown that the conversion of vitamin D_3 to 25-HCC and of 25-HCC to 1,25-DHCC proceeds normally and 1,25-DHCC localizes normally in the intestinal cell nuclei during treatment with cortisone[62]. It has therefore been suggested that steroids inhibit vitamin D-stimulated intestinal Ca absorption by directly affecting the calcium transport mechanism rather than by alteration of vitamin D metabolism.

Hypercalcemia and hypercalciuria may occur in sarcoidosis, probably due to an intestinal hyperabsorption of calcium[64,65]. Hypercalcemia may appear following administration of relatively small doses of vitamin D[66]. It

Circulating Levels of 1α,25-$(OH)_2$-D_3 in Renal Patients as Determined by Radioreceptor Assay

Group	Patient (treatment)	Plasma 1α,25-$(OH)_2$-D_3 (ng/100 ml)	Average 1α,25-$(OH)_2$-D_3 (ng/100 ml ± SD)
Normal	16 total	2.6 - 5.8	4.1 ± 1.0
Renal Failure (untreated)	1	1.3	1.3 ± 0.5*
	2	0.8	
	3	1.7	
Renal Failure (Hemodialysis)	4 (4 yr)	0.1	0.3 ± 0.2†
	5 (2.7 yr)	0.3	
	6 (1.6 yr)	0.1	
	7 (0.2 yr)	0.5	
	8 (1.0 yr)	0.6	
Anephric	9 (2 mo)	0.8	0.5 ± 0.3†
	10 (4 mo)	0.2	
	11 (1 wk)	0.4	
Renal Transplant	10 (3 wks post transplant)	6.0	

Fig. 25

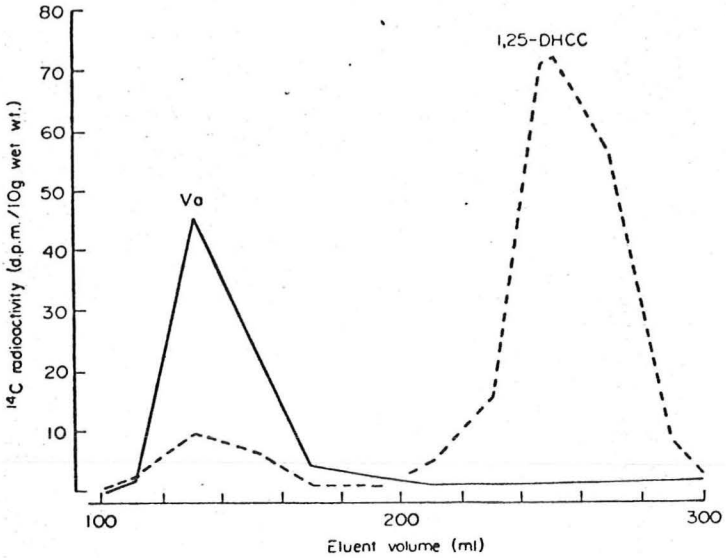


Metabolic balance study in patient with creatinine clearance of 5 to 10 ml/min and clinically overt osteomalacia. Patient received 1,25-$(OH)_2D_3$ at 2.7 µg/day for 6 days. Observations are depicted as in Reifenstein, Albright and Wells with fecal losses as solid columns and urinary excretion as open columns.

Fig. 26

may be aggravated by exposure to sunlight[67], and ameliorated by avoidance of µV light[68] and low Ca diet. Carbohydrate-active steroids may promptly correct both the hypercalcemia and hypercalciuria[64,65] (Fig. 29). Plasma antirachitic activity[65] and concentration of 25-HCC[69] have been shown to be normal. 25-HCC has been shown to convert normally to 1,25-DHCC[70]. Thus, it has been suggested that there is an enhanced sensitivity to vitamin D in sarcoidosis.

In absorptive hypercalciuria[71], an intestinal hyperabsorption is present in association with hypercalciuria and parathyroid suppression. It has therefore been suggested that this condition is characterized by a similar "hypersensitivity to vitamin D" as in sarcoidosis. However, our recent studies in absorptive hypercalciuria indicate that circulating concentration of 1,25-DHCC is not elevated, there is no evidence of bone disease, and that intestinal hyperabsorption of calcium and hypercalciuria are not corrected by steroids.



Sephadex chromatography of peak V metabolites from kidneys of EHDP- and saline-treated (control) rats, 7 days after intrajugular injection of 75 ng of cholecalciferol ($^3H/^{14}C$ = 4). $^3H/^{14}C$ ratio: peak Va = 3·9; 1,25-dihydroxycholecalciferol (1,25-DHCC) = 1·9; 20 ml fractions were collected, with chloroform–hexane (13:7, v/v) as eluent. ——, EHDP; – – – –, saline.

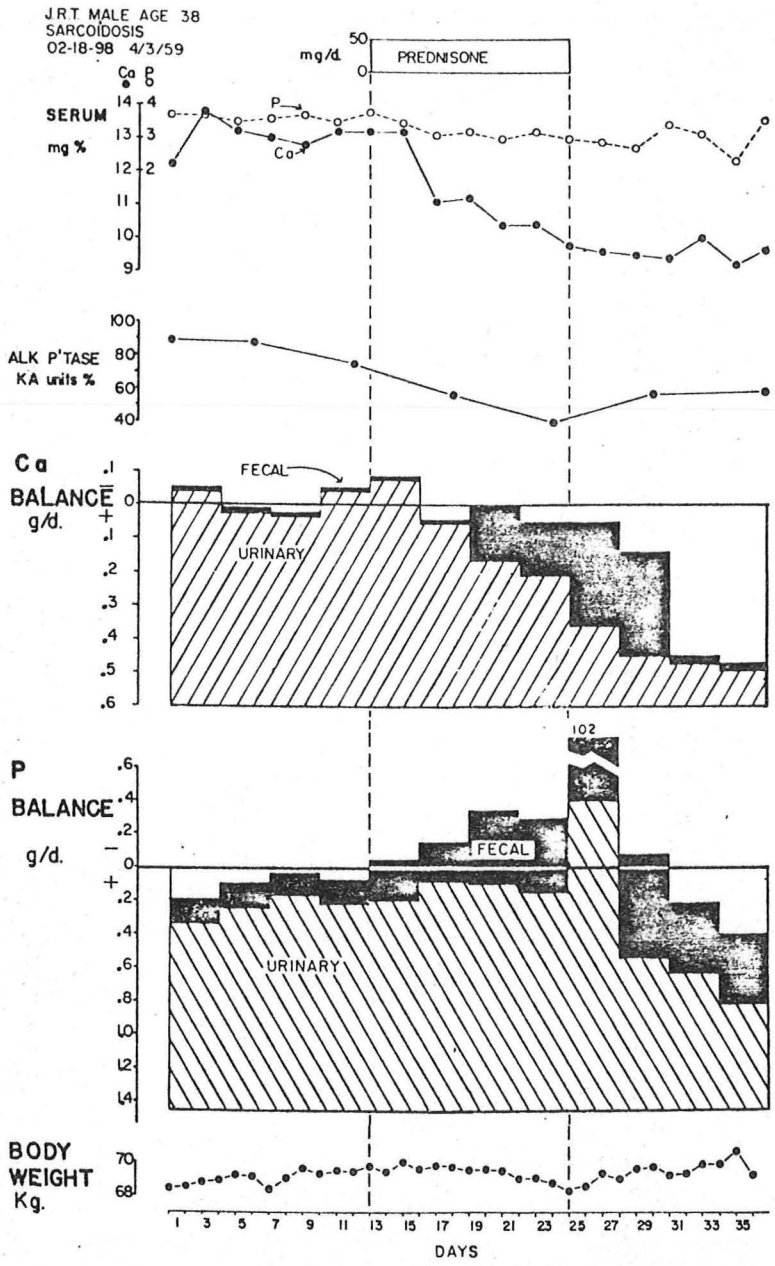
Fig. 27

Stimulation of calcium absorption in EHDP-pretreated rats by 1,25-dihydroxycholecalciferol

Pretreatment	NaCl vehicle	NaCl vehicle	EHDP	EHDP	EHDP	EHDP
Injection on the 8th day	Ethanol vehicle	1,25-$(OH)_2D_3$[a]	Ethanol vehicle	D_3[a]	25-OHD_3[a]	1,25-$(OH)_2D_3$[a]
Experiment I						
Number of animals	4	4	4	—	—	3
Mean calcium absorbed (%)	80.5	87.1	46.8[c]	—	—	82.6
SEM	±2.8	±0.4	±3.1	—	—	±2.7
Experiment II						
Number of animals	4	—	—	4	4	4
Mean calcium absorbed (%)	67.6	—	—	38.9[c]	42.8[b]	74.0
SEM	±3.2	—	—	±1.2	±3.4	±4.9

[a] 325 pmoles intravenously.
[b] $p<0.01$; [c] $p<0.001$ as compared to the corresponding group pretreated with NaCl and injected with ethanol only.
Calcium absorption was measured by the *in situ* duodenal loop method as described in the text.

Fig. 28



The effects of prednisone on serum calcium, phosphorus, and alkaline phosphatase and on calcium and phosphorus balances and body weight in Patient J. T. before onset of nephritis.

Fig. 29

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